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THE EFFECT OF ULTRAVIOLET RADIATION
AND Di-1-p-MENTHENE ON THE PHYTOTOXI-
CITY OF 2,4-D APPLICATIONS TO BLACK
VALENTINE BEAN PLANTS

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PREFACE

The work described in this report was authorized under Task W662605AD2801, Vegetation Control Technology. This work was started in October 1971 and completed in September 1973. The experimental data are recorded in notebook 8673.

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THE EFFECT OF ULTRAVIOLET RADIATION AND DI-1-P-MENTHENE ON THE PHYTOTOXICITY
OF 2,4-D APPLICATIONS TO BLACK VALENTINE BEAN PLANTS

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ABSTRACT

Studies were conducted to determine the relationships, if any, between ultraviolet light, plant growth and plant response to di-1-p-menthene (a dimer of β -pinene), the dimethylamine salt of (2,4-dichlorophenoxy)acetic acid, and the combination of the latter two. Greenhouse-grown bean plants (*Phaseolus vulgaris* L. cv. Black Valentine) were exposed for various time periods to a mid-ultraviolet light source having a major emission peak at 254 nm. The energy level recorded at plant height was 350 ergs/cm²/sec. Plants exposed to this light source for 1 hr had chlorotic, malformed leaves. Plants exposed for longer periods (2 hr) had desiccated, discolored leaves, while plants exposed for 3 hr died. An exposure time of 15 min was used as the maximum safe dose for the subsequent studies in which the plants were exposed to the light source 30 min after application of the herbicide treatments. Irradiation of the plants treated with 2,4-D did not result in a significant reduction in herbicide efficacy. The addition of di-1-p-menthene to the spray solution caused significant ($P \leq 0.05$) increases in the phytotoxicity of the herbicide. This was true irrespective of whether the herbicide application was followed by an exposure to ultraviolet light. The increase in effectiveness of the 2,4-D treatments from the addition of the β -pinene polymer appeared to be synergistic rather than additive.

INTRODUCTION

Previous investigations (13) established that the phytotoxicity of 2,4-D could be increased by combining the herbicide with di-1-p-menthene (a specific formulation of Pinolene^R containing dimers of β -pinene marketed as Nu-Film 17, but hereafter referred to as Pinolene).^{3/} Pinolene is a nontoxic, short-chain polymer which is derived from pine resins. When sprayed on the leaf surface and exposed to light and air, this compound undergoes a slow polymerization to form longer chain polymers (3). It has been studied for use as an antitranspirant (1,2) and has been shown to effectively block stomatal openings. Albrigo (1) reported that the polymer forms a film on older leaves but appears as droplets on younger leaves of orange trees. The droplet formation on the younger leaves was attributed to a low affinity of the polymer for the immature cuticle. Blazquez (5) has studied the ability of Pinolene to prevent decomposition of carbaryl. The addition of Pinolene to the spray mixture was reported to increase the initial deposition of the insecticide on tomato leaves and reduced the rate of decay on the foliage.

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^{3/} Distributed by Miller Chemical and Fertilizer Corp., Hanover, PA 17331.

Information supplied by the manufacturer (3) indicates that this reaction may be photocatalyzed by ultraviolet radiation.

Ordinary window glass admits light in the visible spectrum but prevents the entrance of radiation below 390 nm. Consequently, plants grown in the greenhouse receive little, if any, ultraviolet radiation. It has been known for some time that exposure to radiation of the ultraviolet wavelength may affect biological systems and pesticides. Many common herbicides exhibit their principal electronic absorption maxima in the ultraviolet region (200-400 nm) (7) and photodecomposition by ultraviolet light has been shown to occur in a number of pesticides (4,8,12) including 2,4-D (9). The latter was found to decompose rapidly in the presence of water and ultraviolet irradiation at a wavelength of 254 nm.

Plant growth has also been found to be affected by ultraviolet radiation (6,10,17,18). Dustin (11) noted a "bronzing" of green portions of apple fruits exposed to ultraviolet irradiation. This response is similar to the ultraviolet-induced destruction of chlorophyll noted by other investigations (15). Tranquillini (16) reviewed the work of several investigators on the growth of plants at high altitudes as affected by ultraviolet radiation. Supplemental ultraviolet radiation was found to adversely affect plants from low altitudes and caused death in some species. The plants found at high altitudes were resistant to ultraviolet. Since Pinolene was reported to require ultraviolet radiation for photopolymerization (3), experiments were conducted to determine the relationships, if any, between ultraviolet light, plant growth and plant response to Pinolene, 2,4-D, and Pinolene plus 2,4-D combinations.

MATERIALS AND METHODS

All plants used in these studies were grown from seed sown in 0.95-liter plastic containers filled with standard greenhouse soil. Plants were maintained in the greenhouse where temperatures ranged from 21 to 25 C and relative humidity from 30 to 40%. All treatments were applied to plants 18 days (Experiment I) or 20 days (Experiment II) after planting (14 or 16 days after emergence, respectively).

Ultraviolet radiation was supplied by a bank of mercury-line chromatographic lamps having a major emission peak of 254 nm (2540 Å). However, these lamps also provide some near-ultraviolet light in the 300 to 400 nm region. Photopolymerization of Pinolene has been reported to occur at 285 to 290 nm (3). The spectrum of natural sunlight does not extend below the mid-ultraviolet region at 286 nm (7). Consequently, the ultraviolet lamp source used in these experiments would be expected to supply radiation of the proper wavelength for photopolymerization of Pinolene and would also include particulate waves of a higher frequency than is normally encountered by the plant under natural condition. The light bank consisted of six Westinghouse Steri-lamps (G-15T8) suspended 1 m above the bench top (40 cm from plant tops). This light source supplied 350 ergs per cm² per sec (0.35 mw per cm²) of energy 40 cm above the bench top (plant height).

In Experiment I, bean plants, with and without Pinolene, were exposed for various time periods (1, 2 and 3 hr) to the described lamps to determine if ultraviolet radiation might adversely affect plant growth under the conditions specified. Each treatment consisted of two replications of two plants per pot. All plants were examined daily for visual symptoms of injury. The plants were harvested 7 days after treatment and fresh weights of tops were recorded.

In Experiment II, plants were treated with Pinolene (9.35 L/ha; 1 gal/A), 2,4-D (0.056 kg ae/ha; 0.05 lb ae/A) and a combination of Pinolene and 2,4-D (9.35 L/ha + 0.056 kg ae/ha). The formulation of 2,4-D contained an adjuvant prior to the addition of the Pinolene additive. Treatments were applied in a ventilated spray chamber with a DeVilbiss No. 163 sprayer at 0.1 g/sq cm. The total volume of aqueous spray was equivalent to 355 L/ha. All treated plants were returned to the greenhouse after spray applications. The average time for the droplets to visually dry on the surface of the leaves was 19 min. Approximately 30 min after treatment, one-half of each treatment group (seven replications of one plant per pot) including controls was moved into the room containing the ultraviolet light source and irradiated for 15 min. The plants were then returned to the greenhouse and randomized on the bench with the non-irradiated plants. Plant heights (distance from the cotyledonary node to the terminal bud) were immediately recorded for all plants. Seven and 12 days after treatment, plant heights were again recorded. After the final height measurements on the twelfth day, the plants were harvested as in Experiment I with fresh and dry weight determinations. We then obtained true growth in height over the treatment period for each plant by subtracting its height on the day of treatment.

RESULTS AND DISCUSSION

Under the conditions specified, ultraviolet radiation was found to significantly inhibit growth of Black Valentine bean plants (Figure 1). Symptoms of plants exposed to 60 min of ultraviolet radiation were manifested as malformed leaves with leaflets of unequal size and shape. There appeared to be considerable destruction of chlorophyll, particularly in cells surrounding the vascular tissue. Tissue near the veins was characterized by a light copper coloration extending 5 mm on either side of the major veins. Intervinal tissue did not appear to be affected in that it was normal in coloration and appearance. Since the interveinal tissue represents that part of the lamina which is laid down last in the ontogeny of the leaf, it is possible that this tissue was not present at the time of treatment and hence remained unaffected. Plants exposed to longer periods (120 min) had desiccated, discolored leaves, while leaves of plants exposed for 180 min were dead and overall plant growth was severely retarded (80% inhibition of fresh weight of tops, Figure 1).

Based on the results of Experiment I, it was concluded that long exposure of plants to this ultraviolet lamp system would be undesirable since any herbicidal effects on treated plants would be confounded by the deleterious effects of the ultraviolet radiation. Consequently, an exposure time of 15 min was selected as the maximum dose to use for photopolymerization of the Pinolene additive in the spray solution.

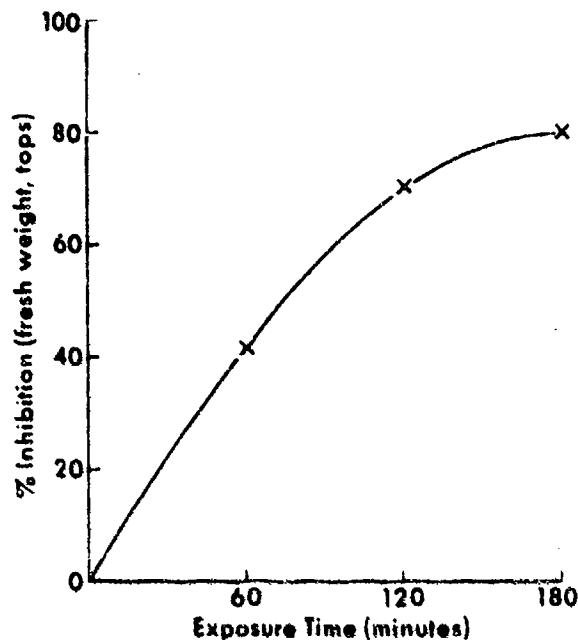


Figure 1. The effect of various exposure times of ultraviolet radiation (254 nm) on growth of Black Valentine bean plants. The radiation level was 350 erg per cm² per sec at plant height.

As we previously demonstrated (13), the addition of Pinolene to an aqueous formulation of the dimethylamine salt of 2,4-D increased the phytotoxicity of the herbicide when it was applied at a sublethal rate (Table 1). Application of 2,4-D alone caused a 16% inhibition of growth in height over the 12-day treatment period, but the combination of 2,4-D and Pinolene caused an 83% inhibition in growth. This was an approximate five-fold increase in phytotoxicity which was significant at $P \leq 0.05$. Using the dry weight data as a criterion of comparative effectiveness, the addition of Pinolene to the spray solution caused an approximate 40% increase in degree of growth suppression (20.2% vs 33.0%). This increase was significant at $P \leq 0.1$ but was not significant at $P \leq 0.05$.

In general, both nontreated and treated plants irradiated for 15 min with ultraviolet light appeared smaller than those not exposed to the ultraviolet light source. With one exception the differences in growth values in Table 1 between irradiated and nonirradiated plants were not significant. The significant difference in inhibition in growth due to exposure to ultraviolet light occurred in the height data for the plants treated with Pinolene alone. This anomaly, however, was not reflected by a corresponding significant difference in dry weight. Growth in height appeared to be a more sensitive criterion of ultraviolet light injury than did suppression of dry weight production, although this does not explain the apparent Pinolene-ultraviolet light interaction.

Table 1. Effect of presence or absence of supplemental ultraviolet light (254 nm)^{1/} on response of Black Valentine bean plants to 9.35 L/ha of Pinolene, 0.056 kg ae/ha of 2,4-D and 2,4-D plus Pinolene. Treatments were foliarly-applied as aqueous sprays equivalent to a total volume of 355 L/ha.

Treatments	No ultraviolet light		Plus ultraviolet light	
	Measurement ^{2/}	% Inhib.	Measurement	% Inhib.
12-Day growth in height (Δ cm)				
Control	45.43 a	0	39.71 ab	0
Pinolene	45.64 a	- 0.5	35.71 b	10.1
2,4-D	38.21 ab	15.9	38.67 ab	2.6
2,4-D + Pinolene	7.86 c	82.7	5.90 c	85.2
Dry wt of tops (g)				
Control	2.97 a	0	2.72 ab	0
Pinolene	2.85 ab	4.0	2.72 abc	0
2,4-D	2.37 abc	20.2	2.25 bc	17.3
2,4-D + Pinolene	1.99 c	33.0	1.94 c	28.7

^{1/} One-half of the plants were exposed for 15 min at foliage level to 350 ergs/cm²/sec of UV light following the chemical treatments. One-half of the controls were similarly exposed to the UV light. The nonultraviolet plants were placed in the greenhouse immediately following application of the chemical treatments.

^{2/} Values are the means of seven replicates. Means followed by the same letters are not significantly different at $P \leq 0.05$, as computed by 95% confidence limits ($\bar{X} \pm t_{0.05} S_{\bar{X}}$).

As shown in Table 1, plants treated with 2,4-D alone and subsequently exposed to ultraviolet light grew within less than one-half centimeter as much as the plants similarly treated but not irradiated (38.21 cm vs 38.67 cm). However, the respective percent inhibitions in growth for these two treatments suggest some loss in efficacy of 2,4-D attributable to ultraviolet light even though the differences are not significant. This apparent difference is a result of calculating these two percent inhibitions relative to their respective controls. The irradiated control was inhibited ca 13% in growth when compared to the nonirradiated control. Using the dry weight data in Table 1 to evaluate the effect of ultraviolet light on the phytotoxicity of the 2,4-D treatments, it can again be seen that there are no real differences in efficacy. Similarly, the presence or absence of ultraviolet light could not be shown to affect the plant's subsequent growth responses to the 2,4-D + Pinolene combination. Both sets of percent inhibition values for growth in height and dry weight were in extremely close agreement, i.e., 82.7% vs 85.2% and 33.0% vs 28.7%. As was found with the nonirradiated group of treatments, the addition of Pinolene to the spray solution markedly increased the phytotoxicity of the 2,4-D treatments.

Since these data show no significant effect on plant response attributable to ultraviolet light per se, it would appear that the photodegradation of 2,4-D by ultraviolet radiation in the literature (9) may be somewhat overstated. Our failure to find a significant loss in the activity of 2,4-D due to ultraviolet light is supported by the work of Penfound and Minyard (14) who studied the effects of 2,4-D on plants grown in sunlight and shade. Although they observed a variable response of water hyacinth plants to phenoxy herbicides attributed to sunlight, repetition of the experiment with potted Red Kidney beans revealed that 2,4-D caused a similar plant response that was independent of where the plants were grown, including darkness.

From these experiments it would appear that the potentiating effect of Pinolene on plant responses to sublethal concentrations of 2,4-D does not require the formation of ultraviolet-mediated polymerized di-l-p-menthene. No visual differences could be seen with a hand lens in the films and droplet residues on the leaves between the irradiated and nonirradiated plants. Since the 2,4-D formulation used in these studies contained a wetting agent, it seems unlikely that Pinolene acted simply as another surfactant. If this were, in fact, true, the increase in phytotoxicity should have been additive rather than synergistic. Pinolene may have simply acted as a sticker or extender, retaining the 2,4-D on the leaf surface for a longer period of time in a semi-liquid form, thereby allowing more time for foliar absorption.

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